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The Cell Cycle in Early Embryonic Development

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ABSTRACT

The cell cycle in early cleavage stages of sea urchin embryos is reviewed. The embryonic cell cycle consists of S, G_2 and M (there is no G_1). Various aspects of mitotic apparatus formation, chromosome movement and the cleavage furrow are discussed. Cell cycle regulation is also reviewed, including factors involved in the control of chromosome condensation cycles and also in terms of cell cycle timing.

The eukaryotic cell cycle represents all of the events occurring in sequence from one cellular division to the next. Cells repetitively undergoing the series of events leading to division are termed cycling cells, while those cells involved in other aspects of development, growth or maintenance (for example, terminally differentiated cells) are considered to be in a non-cycling state. The mature, unfertilized sea urchin egg is one cell which is in a resting or non-cycling state. However, when the egg is fertilized and becomes metabolically activated, the first cell cycle is initiated, unleashing in the egg an awesome potential for division that can result in the production of as many as a thousand cells in 8 hours.[1] Add to this division potential the attraction of being able to fertilize an entire population of cells in such a way that all of the cells within the population divide synchronously, and the sea urchin egg becomes a useful model system for cell cycle studies. The purpose of this discussion is to review some of the events of the sea urchin cell cycle, and some of the proposed mechanisms by which the tempo of the cell cycle may be regulated. Cell cycle models derived from a study of this system must be extrapolated to other cellular systems with care. The egg is, after all, a developmental system, with a requirement for large numbers of divisions over very short time spans, with little or no interphase pause between successive cell divisions.

The basic eukaryotic cell cycle is generally subdivided into four phases designated as G_1 (Gap 1), S, G_2 (Gap 2) and M. In this scheme (Figure 1), interphase (encompassing G_1, S and G_2) is actually a dynamic state of the growing, metabolically active cell. The G_1 phase marks the interval occurring between the end of mitosis (M) and the onset of the DNA synthetic phase (S). It

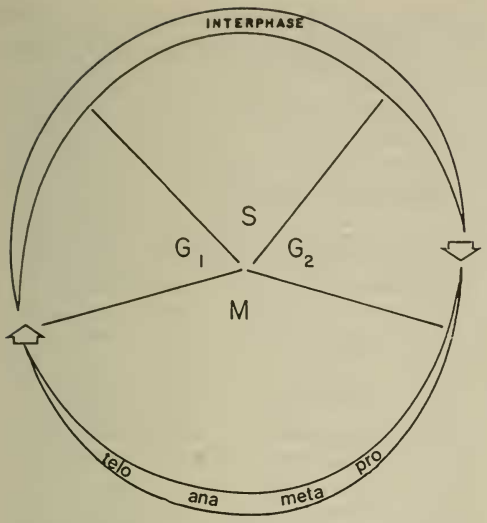

Fig. 1. The normal sequence of the eukaryotic cell cycle. Interphase includes Gap 1 (G_1), DNA synthesis (S) and Gap 2 (G_2). Mitosis (M) includes normal mitotic chromosome movements designated as prophase (pro), metaphase (meta), anaphase (ana) and telophase (telo).

is during G_1 that synthesis and mobilization of the various elements required for DNA synthesis occurs.[2] The S phase represents the period when DNA replication occurs,[3] while G_2 is a relatively nonvariable interval occurring from S to mitotic onset.[2] Mitosis consists of the typical chromosomal stages ensuing as the cell progresses in an orderly manner from prophase through telophase.[2] Also associated with the cycle phases on a temporal basis are protein synthesis, beginning in telophase and lasting through prophase, and RNA synthesis, occurring in late telophase or early interphase (reviewed in[2-4]).

In early sea urchin cleavage stages the typical cell cycle phases are not distinct. Hinegardner *et al.*[5] monitored the cell cycle in fertilized eggs of the purple sea urchin, *Strongylocentrotus purpuratus,* using tritium (^3H) labelled thymidine experiments. The ^3H-thymidine is a specific precursor of chromosomal DNA[6] and its incorporation into newly synthesized DNA can be monitored by typical radioactive assay methods. Using this approach, the first period of DNA synthesis (S_1) is shown to occur at approximately 30 minutes post-fertilization, about the time of pronuclear fusion. Synthetic periods after this initial S phase begin in telophase and end as 'interphase' begins. The overlap of S with M indicates there is an absence of G_1.[5,7] Interphase is very short in these early cleavage divisions (20 minutes in *S. purpuratus* at 15°C[5]) and not well defined.[7] A recent study by Dan *et al.*[7] has further clarified the cell cycle of early cleavage stages in fertilized sea urchin eggs. Using ^3H-thymidine labelling of eggs from the Japanese sea urchin, *Hemicentrotus pulcherrimus*, they verified the cell cycle consists of S, M and a questionable G_2 phase. In this species also, S overlaps with the preceding M, beginning when the chromosomes are still in anaphase and continuing through telophase (Figure 2).

The M phase is the best characterized portion of the sea urchin cell cycle, at least from an ultrastructural and morphological standpoint. During this time chromosomes

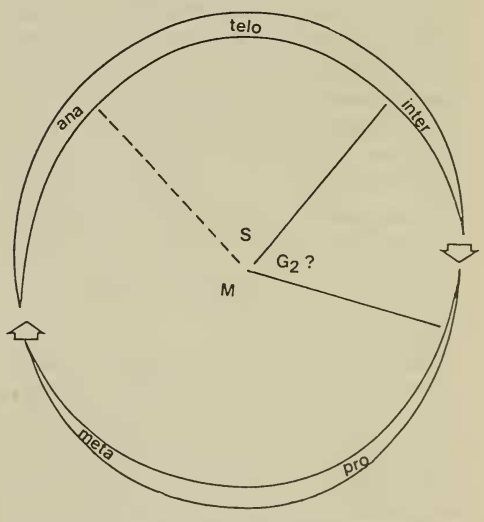

Fig. 2. The sea urchin cell cycle during early cleavage stages. Interphase (inter) is Gap 2 (G_2). Mitosis (M) consists of prophase (pro), metaphase (meta), anaphase (ana) and telophase (telo). The DNA synthetic phase (s) overlaps with M, beginning in late ana or early telo. There is no G_1.

become visibly condensed and associated with other mitotic structures, such as the spindle and mitotic asters. The differentiation of the mitotic apparatus (the chromosomes, spindle and mitotic asters) of the first cell cycle has been described in detail.[8,9,10]

From the time of fertilization and for a period prior to the onset of first prophase, the egg remains in interphase. Following pronuclear fusion, the paternal chromatin at first remains visible as a ball of chromatin, but eventually disappears as it diffuses and mixes with the maternal chromatin.[8] Also prior to the onset of prophase, some cytoplasmic elements (composed of annulate lamelli, ribosomes, smooth endoplasmic reticulum and microtubules[9,11]) concentrate adjacent to the zygote nucleus resulting in the appearance of a 'streak' on the surface of the egg.[11] The chromatin becomes visibly condensed as the cell begins early prophase. The chromosomes continue to contract and condense during prophase[2,12] and the nuclear envelope begins to vesiculate and breakdown (prometaphase[2,9]). At metaphase the chromosomes appear small and have become equatorially arranged. Many microtubules are observed among the chromosomes.[9] The sister chromatids then begin separating toward opposite poles as anaphase is initiated.[2,12] There is no distinct transition from anaphase to telophase.[9] As chromosomes begin to enlarge and disperse, vesicles form about decondensing regions of chromatin, eventually fusing to form a new interphase nucleus.[2,9,12]

Temporally paralleling these events is formation of the sea urchin mitotic apparatus—the cellular structure postulated to function in providing the motive force by which chromosomes move during anaphase. This mitotic apparatus is represented by the mitotic spindle, composed of microtubules, vesicles and possibly actin (reviewed by McIntosh *et al.*[13]) and its associated asters (microtubules, smooth endoplasmic reticulum[8,14]). Formation of the aster and spindle has been studied by both ultrastructural and immunofluorescent means[9,10,14,15]). The developing sperm aster can be seen as early as 13 minutes after fertilization.[10] During male pronuclear migration, microtubules and smooth endoplasmic reticulum begin to surround the sperm centrioles, forming a dense matrix termed the centrosphere. As the sperm pronucleus continues to differentiate, the asters increase in size as microtubules radiate outward from the centrioles.[8] Shortly before pronuclear fusion, a radially arranged system of microtubules is apparent about the egg nucleus,[10] and about the time of pronuclear fusion this system contributes, with the sperm aster, to form the egg monaster.[15] This radial arrangement of fibers is gradually lost as the fibers appear to move to a position underlying and parallel to the egg surface, forming a cortical spiral array.[10,16] This cortical array persists from just prior to pronuclear fusion until the 'streak' stage.[16] During this time, the growing interphase asters become visible, located at either pole of the nucleus,[1] and appearing to increase in size independently of the cortical fiber array.[10] Subsequent to the streak stage, the interphase asters loose microtubules in a progressive manner from the center. A short while later, mitotic aster formation begins as microtubules accumulate and grow from the center outward.[10] These mitotic asters will continue to grow and eventually move to opposite poles of the egg as spindle development occurs. The spindle is also composed of microtubular elements associated with a variety of proteins, endoplasmic reticulum, enzymes, and vesicles,[14,17–19] and provides the pathway along which chromosomes migrate during their anaphase movements.

One intriguing aspect of mitotic nuclear division is the motive force underlying chromosome movement during anaphase. Most models, derived from studies using either lysed cells or the isolated mitotic apparatus, identify spindle microtubules as the force-generating structures.[13, 19–21] Actin

has also been postulated to function in the control of chromosome movement (see review by Forer[22]) but the involvement of actin in the spindle is not well defined. It is now generally accepted that chromosome movement is regulated at least in part by the controlled assembly and dissassembly of microtubule subunits.[20-23]

Upon the completion of M phase the cell is ready to divide, and this is accomplished by the circumferential constriction of the cell to form two new daughter cells (cytokinesis). This constriction is referred to as the cleavage furrow. Cytokinesis usually occurs at telophase, although cleavage furrow formation begins in anaphase.[9,24] The furrow consists of microfilaments which form a contractile ring around the cell margin halfway between the spindle poles.[9,24-26] Cytokinesis is thought to result from the active contraction of cortical substances localized in the furrow.[27] Since the cleavage plane usually coincides with the plane of the metaphase plate, it has been suggested that aster microtubules are involved in stimulating the cleavage furrow. However, Asnes and Schroeder[26] have demonstrated that aster microtubules do not penetrate the equatorial cortex soon enough or in great enough numbers to stimulate the presumptive furrow to constrict, and the mechanism of constriction remains relatively poorly understood.

Most of the cell cycle events discussed are typical of the first five cleavage stages in sea urchin embryos. It is during the early stages (divisions 1–4) that equal division occurs, producing uniformly sized blastomers.[11] It is also during this portion of embryonic development that a high degree of division synchrony among blastomeres is apparent.[5,7] At the fourth cell division, cleavage becomes unequal and embryos containing three cell types separable based on size (micromeres, mesomeres and macromeres) are formed.[11] A study of division synchrony in cleavages after the fifth suggests an overall increase in cycle duration, and increasing asynchronies in cycle times among the three main cell types (although synchrony appears to remain relatively high within a particular cell type[1,7]). Asynchronies among cell types are probably a result of differences in the length of the G_1 and G_2 phases of the cycle, which become incorporated into the cell cycle during these later divisions.

While the sequence of morphological and metabolic events occurring during early cell divisions in sea urchin embryos has been described extensively, the division cycle has historically been less well studied in terms of its regulation. Only in the past few years has the sea urchin egg come into vogue as a model for cell cycle control studies. Most studies using sea urchin eggs as a model system have approached the problem of cycle regulation by asking two basic questions: 1) What process triggers chromosome condensation; and 2) What factors are involved in cell cycle timing?

Cell cycles in sea urchin eggs are usually studied in eggs which have been activated artificially. By treating the eggs with certain chemicals (particularly those of the amine group) several of the activation events normally initiated naturally by fertilization are induced in the absence of any sperm contact.[28-32] One useful aspect of artificial activation is that eggs treated in this manner can still be fertilized and will develop into normal larval forms,[33] although care must be taken to use sperm concentrations which reduce the risk of polyspermy (the incorporation of more than one sperm per egg). Because various cycle events are initiated by the activators before sperm contact, fertilization can be made to occur at various points within the cell cycle simply by varying the length of treatment time before fertilization. By fertilizing the eggs during different portions of the cell cycle, and then determining the effect on various cycle events (such as paternal chromosome condensation, the length of the cell cycle relative to control eggs, etc.) one can address the question of phase dependencies of various cleavage related events on spe-

cific cell cycle events. An alternative approach to the study of the interdependencies of various cycle events is to study division timing after the addition of specific metabolic inhibitors which affect one or more cycle related events.

1. The Control of Chromosome Condensation Cycles

The control of chromosome condensation in sea urchin eggs can be studied by cell fusion involving either egg–sperm interactions[34,35] or by egg–egg hybridization techniques.[36] In both approaches, the results are the same. Fusion of a cell in interphase with a cell in mitosis results in stimulation of chromosome condensation in the interphase cells.

Treatment of unfertilized eggs with ammonia stimulates ^{3}H-thymidine incorporation (DNA synthesis), and eggs treated for extended periods of time display cycles of ^{3}H-thymidine incorporation representative of repetitive S phases of the cell cycle.[32] Even though successive chromosome cycles occur, there is no spindle formation, and normal anaphase movements do not occur.[37] When these eggs are treated for approximately 60 minutes, and then fertilized, both the maternal and paternal chromatin is visibly condensed by 90 minutes post-activation.[34] The maternal chromosomes are condensing at the expected time (90 minutes post-activation), but the paternal chromatin is condensing an hour prematurely (30 minutes post-insemination).[34,35,38] Here fusion of sperm with an egg already into the condensation cycle induces premature condensation (PCC) of the paternal chromatin. Poccia *et al.*[38] analyzed this response of the male chromatin in an activated, cycling egg further in an attempt to determine whether cytoplasmic factors might be involved in controlling PCC. When whole, activated eggs are fertilized after maternal chromosomes have entered prophase, the paternal chromatin condensation cycle conforms to that of the maternal pronuclear cycle. If unfertilized eggs are broken into nucleated and non-nucleated egg halves (a technique performed by centrifugation of the eggs through sucrose gradients[11,39]) and treated with ammonia, both eggs activate,[40] and chromosome cycles are visible in the nucleated halves. If the halves are fertilized when the nucleated halves are in prophase, both halves show PCC at the same time, but non-nucleated halves do not give as strong a response, and the lifetime of PCC-promoting activity is less than in nucleated halves.[35,38] It seems from these results that cytoplasmic components effect PCC-promoting activity, but these effects are modulated by the maternal pronucleus.[38]

Vacquier and Brandriff[40] studied the effects of the activator, procaine hydrochloride, on chromosome cycles and on S. Procaine, like NH_4Cl, stimulates late phase fertilization events, and also induces cytaster formation,[41] the assembly and disassembly of which follows the procaine induced chromosome cycle.[42] Vacquier and Brandriff show that DNA synthesis can be turned on and off by the addition and removal of procaine. Also, when procaine is removed by washing the treated eggs with fresh sea water after chromosome condensation has begun, chromosomes continue to condense, but do not decondense.[41] These workers suggest it is chromosome decondensation which is controlling entrance into S.

Removal of NH_4OH does not appear to turn off chromosome cycles once they have been initiated.[12] However, removal of NH_4Cl gives procaine-like results. If NH_4Cl is removed after chromosome condensation has started, condensation will proceed, but decondensation does not occur.[43] But, if chromosomes have started to decondense when the NH_4Cl is removed, they will continue to decondense (and may even initiate a second cycle[43]).

Preliminary experiments suggest that

chromosome cycles can be initiated by activating eggs with NH_4Cl in sodium-calcium free sea water,[44] ionic conditions which are supposed to prevent any intracellular increases in calcium concentration at activation.[45] Also, treating sea urchin eggs simultaneously with NH_4Cl and protein synthesis inhibitors in either normal sea water or sodium-calcium free sea water, seems to allow chromosome condensation, but prevents chromosome decondensation.[44]

These activator removal experiments and inhibitor experiments suggest that chromosomes cycles in sea urchins may be biphasic, with each phase (condensation and decondensation) independently regulated by separate metabolic events.[44] It has previously been shown that eggs fertilized in the presence of protein synthesis inhibitors do not cycle and work by Wagenaar and Mazia[46] suggests that cytoplasmic factors regulating chromosome cycles are in fact proteins. They were able to demonstrate that a specific sequence of protein synthesis is required for complete and normal progression of the chromosome cycle and that proteins produced at different times in the cell cycle are required for chromosome condensation, nuclear envelope breakdown, and formation of a normal nuclear mitotic apparatus.[46]

2. Control of Cell Cycle Timing

Sea urchin eggs which have been activated with monogen or duponal, and then fertilized, cleave earlier than untreated, fertilized controls. This decrease in the time to first cleavage is dependent on the length of activator treatment.[47,48] Sea urchin eggs activated with NH_4Cl or procaine show this same time dependency (cleavage advance) relative to control eggs.[43,44] If eggs are fertilized before maternal pronuclear prophase has begun, there is no cleavage advance. However, as eggs begin to initiate chromosome condensation, a short advance occurs, and increases to a maximum at a time when 100% of the eggs contain maximally condensed chromosomes. Cleavage advance remains constant as chromosomes enter the decondensation phases of the cycle, but decreases again at interphase and early prophase stages of the next cycle. This pattern is repeated with successive cycles, and suggests that cleavage advance is dependent on the degree of chromosome condensation, but not decondensation (decondensation is not a factor in cycle timing) and the capacity to cleave in advance is reset with each successive cycle.[43]

It is not likely that the timing of cellular progression is directly controlled by chromosome condensation,[49] and it is also unlikely that cell division advance is due to the physical process of chromosome condensation.[50] Sluder[49] has shown that chromosome cycles in sea urchins will proceed to completion, even in eggs which have been exposed to colcemid (a drug which prevents microtubule assembly, including assembly of spindle microtubules). Eggs fertilized after treatment with colcemid show complete chromosome cycles, but do not form spindles and do not divide. The cell cycle itself is slowed by colcemid treatment, and this delay in the cycle appears to reflect a prolongation of prometaphase.[49] By irradiating colcemid treated eggs, the colcemid is inactivated[49] and spindle assembly occurs, but delayed relative to nuclear envelope breakdown (depending on when after nuclear envelope breakdown irradiation occurs). These delays in spindle assembly are reflected as delays in the initiation of anaphase and in the time of cell division. Based on these results, Sluder suggests that microtubule assembly cycles 'set' the tempo of the cell cycle, forming part of the trigger that turns on the events critical to completion of the cycle subsequent to nuclear envelope breakdown.[49]

It is true that microtubules assemble and dissassemble, and that the pattern of assembly can be correlated with the cell cycle.[51] And it is generally accepted that

microtubules are universally associated with chromosomes during cell division. As mentioned previously, the sea urchin aster is primarily composed of microtubules and smooth endoplasmic reticulum. While microtubules themselves comprise only a small portion of the spindle apparatus (the mitotic gel is actually made up of membrane bound vesicles within which the microtubules are embedded), a close relationship is maintained between these two structures throughout the mitotic cycle.[14] Microtubule assembly is influenced by calcium, and vesicles with calcium ion sequestering activity have been identified in the isolated sea urchin mitotic apparatus.[18] In addition, a calcium activated ATPase has been implicated in the mitotic cycle. Mazia *et al.*[52] identified this enzyme in mitotic apparatus isolated from echinoderm eggs, and Petzelt[53] showed initiation of a calcium-ATPase activity cycle in fertilized sea urchin eggs which can be correlated with the cell cycle. Enzyme activity peaks near the start of chromosome condensation and decreases when nuclear membranes are reformed.[53] Harris[54] postulates that mitosis is controlled by the temporal and spatial effects of calcium on microtubule assembly and dissassembly. This theory suggests that unknown factors (i.e., pH, critical tubulin levels, cAMP, etc.) stimulate a self-propogated trigger wave of calcium release which initiates at the aster center and radiates outward, causing in its path a change in the state of tubulin polymerization. While attributing changes in tubulin polymerization to changes in calcium ion concentration, it is not clear from this model which cytoplasmic microtubule elements are forming mitotic microtubules or what specific event is triggering the initial process (i.e., what is resetting the timer).

It does not appear that a 'central timer' is executing a short lived state which allows cleavage to occur. When chromosome condensation is uncoupled from chromosome decondensation by activator removal cleavage is not affected. As long as chromosomes remain condensed maximal cleavage advance will occur,[44] suggesting some permissive signal to cleavage may be turned on indefinitely in these eggs. Chromosome decondensation, then, may be a reset signal required for cell cycle continuity.[50] A survey of results obtained from studies involving the manipulation of the cell cycle would suggest that a model analogous to Hartwell's dependent pathway model (described for yeast) may be an appropriate model for the sea urchin egg also.[50] Here two or more 'loops' (for example a chromosome loop and a metabolic loop) are running in parallel circuit, and close together at the appropriate time to initiate cleavage.

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